a' cont.

diseases such as cancers, by utilizing a novel polypeptide having β1,3-galactosyltransferase activity.

Please substitute the paragraph starting at page10, line 36 and ending at page 11, line 2 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

---

a²

25. A method for determining the expression level of a gene encoding the polypeptide of any one of items 1 to 4, which comprises hybridization using DNA coding for said polypeptide or a fragment of said DNA.

Please substitute the paragraph at page 11, lines 10-27 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

---

27. A DNA according to item 26 wherein the oligonucleotide derivative is selected from the group consisting of an oligonucleotide derivative in which the phosphodiester bond is converted into a phosphorothioate bond, an oligonucleotide derivative in which the phosphodiester bond is converted into an N3'-P5'-phosphoamidate

a³

bond, an oligonucleotide derivative in which the ribose and the phosphodiester bond are converted into a peptide-nucleic acid bond, an oligonucleotide derivative in which the uracil is replaced by a C-5 propynyluracil, an oligonucleotide derivative in which the uracil is replaced by a C-5 thiazolyluracil, an oligonucleotide derivative in which the cytosine is replaced by a C-5 propynylcytosine, an oligonucleotide derivative in which the cytosine is replaced by a phenoxazine-modified cytosine, an oligonucleotide derivative in which the

a3 cont.

ribose is replaced by a 2'-O-propylribose, and an oligonucleotide derivative in which the ribose is replaced by a 2'-methoxyethoxyribose.

Please substitute the paragraph at page 11, lines 30-33 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a4

29. A method for determining the expression level of a gene encoding the polypeptide of any one of items 1 to 4, which comprises polymerase chain reaction using the DNA of any one of items 26 to 28.

Please substitute the paragraph at page 17, lines 30-33 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a5

The expression vector into which the cDNA has been integrated is introduced into animal cells capable of expressing the objective cDNA, to give transformed cells.

Please substitute the paragraph at page 22, lines 7-26 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a6

The expression vector includes, e.g., pBTrp2, pBTac1, pBTac2 (which all are commercially available from Boehringer Mannheim), pKK233-2 (a product of Pharmacia), pSE280 (a product of Invitrogen), pGEMEX-1 (a product of Promega), pQE-8 (a product of QIAGEN), pKYP10 (Japanese Published Unexamined Patent Application No. 110600/83), pKYP200 [Agric. Biol. Chem., 48, 669 (1984)], pLSA1 [Agric. Biol.

Chem., 53, 277 (1989)], pGEL1 [Proc. Natl. Acad. Sci., USA, 82, 4306 (1985)], pBluescript II SK+ (a product of Stratagene), pBluescript II SK(-) (a product of Stratagene), pTrs30 (FERM BP-5407), pTrs32 (FERM BP-5408), pGHA2 (FERM BP-400), pGKA2 (FERM BP-6798), pTerm2 (Japanese Published Unexamined Patent Application No. 22979/91, US 4686191, US 4939094, US 5160735), pEG400 [J. Bacteriol., 172, 2392 (1990)], pGEX (a product of Pharmacia), pET system (a product of Novagen), pSupex, pUB110, pTP5, pC194, pTrxFus (a product of Invitrogen), pMAL-c2 (a product of New England Biolabs), pUC19 [Gene, 33, 103 (1985)], pSTV28 (a product of Takara Shuzo Co., Ltd.), pUC118 (a product of Takara Shuzo Co., Ltd.) and pPA1 (Japanese Published Unexamined Patent Application No. 233798/88).

a6 cont.

Please substitute the paragraph at page 38, lines 9-23 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

That is, N-acetylglucosamine monosaccharides, oligosaccharides having N-acetylglucosamine residue at the non-reducing termini thereof, or complex carbohydrates having N-acetylglucosamine residue at the non-reducing termini of sugar chains thereof, are used as the acceptor substrate while the polypeptide of the present invention obtained in the method described in item (2) above is used as the enzyme source, and reaction products having galactose transferred via β1,3-linkage to N-acetylglucosamine monosaccharide or N-acetylglucosamine residue of the acceptor substrate can be produced by allowing said receptor substrate, said enzyme source and uridine-5'-diphosphate galactose (UDP-Gal) to

a7

a7 cont.

be present in an aqueous medium to thereby form and accumulate said reaction products in said aqueous medium and collecting said reaction products from said aqueous medium.

Please substitute the paragraph starting at page 44, line 36 and ending at page 45, line 7 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a8

The class and subclass of the antibody are determined using a mouse monoclonal antibody typing kit or a rat monoclonal antibody typing kit. The class of the antibody means isotype of the antibody, and for example, mention can be made of IgG, IgA, IgM,IgD and IgE in human. The subclass of the antibody means isotype in the class, and for example, mention can be made of IgG1, IgG2a, IgG2b and IgG3 in mouse, and IgG1, IgG2, IgG3 and IgG4 in human.

Please substitute the paragraph starting at page 54, line 29 and ending at page 55, line 4 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a9

Fig. 1, A shows the results of measurement of the expression levels of type 1 sugar chains (sialyl-Lewis a sugar chain, Lewis a sugar chain, Lewis b sugar chain) in a wide variety of human cancer cell lines. Each kind of cell was stained with a fluorescent antibody using anti-sialyl-Lewis a sugar chain antibody (19-9), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody(TT42) and then analyzed by FACS. The reactivity with each antibody is shown as +++, ++, +, ±, and –, in the order of decreasing reactivity. – means the absence of reactivity with the antibody. NT means that the analysis was not conducted.

Please substitute the paragraphs at page 55, lines 15-32 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

Fig. 3, A shows the results of indirect fluorescent antibody staining with anti-sialyl-Lewis c sugar chain antibody (DU-PAN-2) and subsequent FACS analysis of Namalwa cells (Namalwa-mock) having the control plasmid (pAMo) introduced therein, or Namalwa cells (Namalwa-3GT5) having human β3Gal-T5 expression plasmid (pAMo-3GT5) introduced therein. The shaded histogram shows the result of analysis using A-PBS in place of DU-PAN-2.

a10

Fig. 3, B shows the results of indirect fluorescent antibody staining with anti-sialyl-Lewis a sugar chain antibody (19-9), anti-sialyl-Lewis c sugar chain antibody (DU-PAN-2), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody (TT42) and subsequent FACS analysis of HCT-15 cells (HCT-mock) having the control plasmid (pAMo) introduced therein, or HCT-15 cells (HCT-3GT5H) having human β3Gal-T5 expression plasmid (pAMo-3GT5) introduced therein. The shaded histogram shows the result of analysis using A-PBS in place of DU-PAN-2.

Please substitute the paragraph at page 56, lines 22-29 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

Fig. 6, C shows the results of examination of the expression level of each isoform of human β3Gal-T5 cDNA in Colo205 cells by the RT-PCR method. After RT-PCR was conducted with the combination of primers shown in Fig. 6, A, the reaction

a11

a11 corrd.

product was cleaved with a restriction enzyme (XbaI or BsmI) shown in Fig. 6, to specify the isoform.(-) means that the restriction enzyme treatment was not conducted. The left line shows molecular markers (100 bp ladder).

Please substitute the paragraph at page 58, lines 8-13 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a12

The cells described above were cultured in a medium suitable for the respective cells, and then the cells were subjected to fluorescent antibody staining with anti-sialyl-Lewis a sugar chain antibody (19-9), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody (TT42) and analyzed by FACS.

Please substitute the paragraph at page 60, lines 16-19 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a13

As the internal controls, linear DNAs were prepared by cleaving plasmids prepared below (pUC119-3GT1d, pBS-3GT2d, pBS-3GT3d, pBS-3GT4d) with suitable restriction enzymes to take the cDNA inserts.

Please substitute the paragraph at page 60, lines 24-25 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a14

By deleting a 183-bp sequence between StyI-StyI in human β3Gal-T3 cDNA in pBS-3GT3, pBS-3GT3d was prepared.

Please substitute the paragraph at page 71, lines 5-10 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a15

The single clone (HCT-3GT5H) thus obtained was subjected to indirect fluorescent antibody staining with anti-sialyl-Lewis a sugar chain antibody (19-9), anti-sialyl-Lewis c sugar chain antibody (DU-PAN-2, a product of Kyowa Medex), anti-Lewis a sugar chain antibody (7LE) or anti-Lewis b sugar chain antibody (TT42).

Please substitute the paragraph at page 73, lines 19-21 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a16

The respective cells ($1x10^7$ cells) were suspended in a solution [20 mmol/l HEPES (pH 7.2), 2% Triton X-100] and sonicated in a short time to prepare a cell lysate.

Please substitute the paragraph at page 76 , lines 1-3 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

a17

Said transformed cells were suspended in a solution [20 mmol/l HEPES (pH 7.2), 2% Triton X-100] and sonicated in a short time to prepare a cell lysate solution.

Please substitute the paragraph at page 76, lines 14-19 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

Specifically, the activity was measured by identifying a product by high performance liquid chromatography (HPLC) after reaction at 37°C for 2 hours in 10 μl assay solution [14 mmol/l HEPES (pH 7.4), 75 μmol/l UDP-Gal (a product of SIGMA), 11 μmol/1 $MnCl_2$, 0.01% Triton X-100, 25 μmol/l pyridylaminated sugar chain substrate, and the above cell lysate solution].

Please substitute the paragraphs at page 79, lines 12-27 with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made thereto, is attached.

The β3Gal-T5 transcripts in various human tissues (brain, lung, esophagus, stomach (body), stomach (antrum), jejunum, colon, liver, pancreas, spleen, kidney, adrenal, uterus, peripheral blood lymphocytes) were quantified by RT-PCR in the same manner as in item (7) in Example 2. The amount of the β3Gal-T5 gene transcript in each kind of organs is shown as a value relative to the amount (= 1000) of the β-actin transcript (Fig. 5).

It was revealed that the β3Gal-T5 transcripts are significantly expressed in the stomach (body), stomach (antrum), jejunum, colon and pancreas. Further, the β3Gal-T5 transcripts were slightly expressed in the brain, esophagus, kidney and uterus. On the other hand, the β3Gal-T5 transcripts were not expressed in the lung, liver, spleen, adrenal and peripheral blood lymphocytes.

REQUEST FOR APPROVAL OF DRAWING CHANGES:

The Examiner is requested to approve the following changes to the drawings: